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Review

Immune Reconstitution after Haploidentical Hematopoietic Stem Cell Transplantation

Ying-Jun Chang [1], Xiao-Yu Zhao [1], Xiao-Jun Huang [1,2,*]

[1] Peking University People's Hospital and Peking University Institute of Hematology, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing, China
[2] Peking-Tsinghua Center for Life Sciences, Beijing, China

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Haploidentical hematopoietic stem cell transplantation (HSCT) offers the benefits of rapid and nearly universal donor availability and has been accepted worldwide as an alternative treatment for patients with hematologic malignancies who do not have a completely HLA-matched sibling or who require urgent transplantation. Unfortunately, serious infections and leukemia relapse resulting from slow immune reconstitution remain the 2 most frequent causes of mortality in patients undergoing haploidentical HSCT, particularly in those receiving extensively T cell–depleted megadose $CD34^+$ allografts. This review summarizes advances in immune recovery after haploidentical HSCT, focusing on the immune subsets likely to have the greatest impact on clinical outcomes. The progress made in accelerating immune reconstitution using different strategies after haploidentical HSCT is also discussed. It is our belief that a predictive immune subset–guided strategy to improve immune recovery might represent a future clinical direction.

INTRODUCTION

Haploidentical hematopoietic stem cell (HSC) transplantation (HSCT) is available for nearly all patients and has no search or acquisition costs [1-9]. Over the past decade, many haploidentical transplantation protocols, including T cell–replete and T cell–depleted (TCD) haplotype HSCT, depending on whether or not allografts have been engineered in vitro, have demonstrated promising clinical outcomes [4,8,9]. Unfortunately, serious infections and disease relapse resulting from delayed immune reconstitution remain the 2 most frequent causes of mortality after allogeneic HSCT, particularly in patients who received extensively TCD $CD34^+$ cell megadose allografts [10-15]. Advances in the understanding of immune recovery profiles in haploidentical recipients [8,16-47], along with new methods of modifying donor T cells [48-50] and natural killer (NK) cells [51], have made it possible to establish new strategies to improve post-transplantation immunologic reconstitution [9,52-58].

In this review, we summarize advances in immune recovery after haploidentical HSCT, focusing on the recovered immune subsets likely to have the greatest impact on clinical outcomes [18,23,33,42,45,59-63]. We compare the differences in immune reconstitution between haploidentical transplantation and other transplantation strategies, including HLA-identical sibling donor transplantation, HLA-matched unrelated donor transplantation, and umbilical cord blood transplantation. In addition, we discuss recent advances in the enhancement of immune reconstitution after haploidentical HSCT [64-70].

KINETICS OF IMMUNE RECOVERY AFTER HAPLOIDENTICAL HSCT

Different immune cell subgroups recover at different rates after haploidentical HSCT (Table 1). The conditioning regimen is followed by a "neutropenic" phase that lasts until neutrophils reconstitute, at a median of approximately 11 to 12 days after TCD HSCT with high doses of $CD34^+$ cells [8,38], approximately 15 days after unmanipulated haploidentical steady-state bone marrow (BM) allografts, approximately 21 days after granulocyte colony-stimulating factor (G-CSF)-primed BM allografts, and approximately 13 days after G-CSF–stimulated blood and BM allografts [2,7,71]. The recovery of neutrophil function (eg, chemotaxis, phagocytosis, superoxide production, killing of bacteria) in haploidentical settings remains poorly understood, however.

The rapid recovery of NK cells after haploidentical transplantation is based on an expansion of the cytokine-producing $CD56^{bright}$ NK cell subsets in both T cell–replete and TCD settings [16,17,24,41]. Although the absolute number of overall NK cells usually recovers to the donor's level by day 30 post-HSCT, recovery may be delayed by the development of graft-versus-host disease (GVHD) [8,24]. The function of NK cell recovery is regulated by the balance of activating and inhibitory signals transmitted by different cell surface receptors. Thus, the expression of activating NK receptors (NKRs), such as NKP46, NKP44, NKP30, and NKG2D, as well as inhibiting NKRs, such as CD158a, CD158b, CD158e, and NKG2A, is essential for NK cell activation.

After haploidentical HSCT, the overall expression of activating NKRs and inhibitory NKRs is reduced, whereas CD94/NKG2A expression is increased. NKG2A recovery is inversely correlated with CD158 recovery in the year after HSCT. This altered phenotype includes more $CD56^{bright}$ cells, fewer $CD56^{dim}$ NK cells, and altered CD94/NKGA expression. Activation or inhibition of NKR expression during early reconstitution is associated with lower levels of in vitro NK

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* Correspondence and reprint requests: Xiao-Jun Huang, MD, Professor, Peking University Institute of Hematology, Peking University People's Hospital, No. 11 Xizhimen South St, Beijing 100044, China.
E-mail address: xjhrm@medmail.com.cn (X.-J. Huang).

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cytotoxicity after haploidentical HSCT. The reconstitution of killer cell immunoglobulin-like receptors (KIRs) is influenced by many factors, including the conditioning regimen, level of T cell depletion, and the use of immune suppression after transplantation, as recently reviewed by Zhao et al. [72]. Monocyte engraftment is rapid, with normal values on day +15 after unmanipulated haploidentical blood and BM transplantation [25]. In HLA-identical HSCT settings, the absolute monocyte counts at day +30 (>300 cells/μL) are strongly associated with improved survival [73,74], whereas no association between monocyte recovery and outcome after haploidentical HSCT has been reported.

Dendritic cells (DCs) are highly important antigen-presenting cells with central roles in initiating and modulating immune responses. Human peripheral blood DCs are divided into 2 major subsets: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). mDCs are further subdivided into 2 subsets: T helper cell (Th) 1-promoting mDCs (mDC1s) and Th2-promoting mDCs (mDC2s) [75]. Our preliminary data showed extremely low mDC1, mDC2, and PDC counts at 1-3 months post-HSCT, which recovered to normal values by 1 year after unmanipulated haploidentical blood and BM transplantation [25]. A gradual increase in the DC population after transplantation was also reported in pediatric patients with acute leukemia who received CD3/CD19-depleted grafts from haploidentical donors [41]. The early delayed immune reconstitution of DCs may contribute in part to an increased rate of postengraftment bacterial and fungal infections [76], although little is known about the recovery of DC function after haploidentical transplantation [25,41].

Invariant NK T (iNKT) cells are a specialized subset of T cells that use their T cell receptors (TCRs) to recognize self and foreign lipids presented by CD1d as cognate antigens. These cells can have protective or harmful roles in many pathological states, including microbial infection, autoimmune disease, allergic disease, and cancer [77]. In pediatric patients, de Lalla et al. [33] observed that iNKT recovered slowly after CD34-selected haploidentical HSCT and reached normal reference values by 18 months post-transplantation. Their data also suggest that the frequency of iNKT cells is significantly correlated with the remission state after haploidentical HSCT. In HLA-identical transplantation settings, Chaidos et al. [78] reported that CD4(-) iNKT cell dose was the sole graft parameter predictive of clinically significant acute GVHD. This interesting finding awaits confirmation in haploidentical HSCT settings.

Detailed analyses of adaptive immune reconstitution have been performed by researchers at various transplantation centers [16,17,22,25-27,29,32,33,38,41,45,79-81]. After CD34-selected haplodentical HSCT, mean (±standard deviation) $CD4^+$ cell counts ranged from 100 ± 40/L to 200 ± 20/L at 10 months post-transplantation, and rose thereafter. The mean $CD8^+$ cell count reached 230 ± 80/L on day +60, followed by a steady rise to 570 ± 125/L by day +300. The mean $CD16^+$ NK cell count reached 400/L stably by day +30 [38]. The recovered NK cells may enhance the graft-versus-leukemia effect [38-82]. A German group used negative selection of CD3/CD19 cells to retain NK cells, monocytes, and DCs in haploidentical allografts and found delayed T cell reconstitution, with a median of 369 $CD3^+$ cells/μL, 177 $CD4^+$ cells/μL, and 193 $CD8^+$ cells/μL on day +400 [8,83]. In our unmanipulated haploidentical blood and BM transplantation protocol, a median absolute number of 277 $CD4^+$ T cells/μL and 884 $CD8^+$ T cells/μL were recovered at 1 year post-transplantation [25]. Immune recovery after unmanipulated haploidentical HSCT appears to be more rapid than CD34-selected haploidentical transplantation; however, the 2 transplantation methods are not strictly comparable [17,25,38,39].

The expansion of memory T cells (especially $CD4^+$ memory T cells, which reconstitute later than $CD8^+$ memory T cells and rely more on thymic production of naive T cells) results in a significant inversion of the CD4/CD8 ratio up to 1 year after transplantation [25,39,41]. In contrast to most previously reported results [25,27,36,38,39,41], no significant inversion of the CD4/CD8 ratio was observed after haploidentical HSCT using reduced-intensity conditioning and CD3/CD19-depleted grafts [17]. In the TCD haploidentical HSCT protocol [45], the main lymphocyte populations (including $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, and B cells) and the CD4/CD8 ratio reconstituted to age-matched control levels by 4 to 6 years post-transplantation. Similar kinetics in B cell recovery have been reported by our group and others [17,25,41,84], with absolute numbers of B cells increasing gradually and reaching normal levels more than 1 year after HSCT.

Regarding the reconstutiton of T cell function, an interesting finding is that the ability of T cells to secrete IFN and IL-4 recovers to normal level by day +30 post-HSCT in patients without acute GVHD, although TCR rearrangement excision DNA circle (TREC) levels remain low for 12 to 24 months after unmanipulated haploidentical HSCT [29]. At 4 to 6 years after TCD haploidentical HSCT, recipients demonstrate higher proportions of $CD31^+$ naïve $CD4^+$ T cells compared with donors [17,45]. The signal-joint TCR excision circle (sjTREC) levels in the recipients tend to be higher than the levels in donors, which are similar to those seen in age-matched control subjects.

The foregoing observations suggest that long-term T cell reconstitution is critically influenced by de novo T cell production, reflecting thymopoiesis as a central mechanism. A skewed and restricted distribution of TCR repertoires has been observed at less than 180 days post-transplantation [17]. Azevedo et al. [45] reported that at 4-6 years post-transplantation, patient $CD4^+$ T cells and $CD8^+$ T cells exhibit a diverse T cell repertoire and reconstitute a diverse regulatory T cell (Treg) pool through a mechanism involving de novo thymic production. Their findings provide strong evidence that a normal immune system can be reconstituted after haploidentical HSCT; however, the small number of patients in their study precluded from a subgroup analysis to identify the factors influencing immune reconstitution. In addition, the function of T cells in unmanipulated haploidentical HSCT remains to be investigated in depth [2,71,85-88].

More recently, Ciurea et al. [36] demonstrated that compared with patients who underwent TCD haploidentical HSCT, patients who received T cell–replete haploidentical allografts exhibit better immune reconstitution of T cell subsets, including $CD4^+$ T cells, $CD8^+$ T cells, naive T cells, and memory T cells, during the first 6 months after transplantation (Table 1). The authors suggested that improved immune reconstitution might contribute to the improved early outcomes observed with the use of T cell–replete grafts [36]. Thus, a prospective study to compare immune recovery between TCD and T cell–replete haploidentical approaches is warranted.

Overall, the first 90 days after haploidentical HSCT are characterized by persistent $CD4^+$ and $CD4^+$ naive T cells, B cell lymphopenia, and low thymic function, which render patients especially susceptible to viral and fungal infections